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ARAŞTIRMA MAKALESİ / **RESEARCH ARTICLE**

**Esra KOÇ[1], Ayşen Sülün ÜSTÜN[1], Cemil İŞLEK[2], Yeliz KAŞKO ARICI[3]**

# EFFECT OF EXOGENOUSLY APPLIED SPERMINE AND PUTRESCINE ON GERMINATION AND *IN VITRO* GROWTH OF PEPPER (*CAPSICUM ANNUUM* L.) SEEDS UNDER SALT STRESS

## *ABSTRACT*

Plant growth regulators play a significant role in germination of seed. In this study, Effects of exogenous polyamines (spermine and putrescine) on germination of seed and seedling growth such as radicula length, hypocotile length, fresh weight, dry weight of pepper under salt stress were investigated. According to results, increased salt concentration resulted in a significant reduction in germination ($P<0.05$). NaCl prevented hypocotile growth and this inhibition was reversed by applying spermine. During application of 2 mM spermine + 200 mM NaCl ($P<0.05$), the best germination was recorded. On the contrary, germination of seed was inhibited by single application of Put but hypocotile and radicula length of seedling were increased seriously by application of 0.01 mM putrescine + 200mM NaCl and 1mM putrescine + 50 mM NaCl ($P<0.05$) in pepper plant.

**Keywords:** Germination, Growth, Pepper, Putrescine, Polyamines, Spermine

# TUZ STRESİ ALTINDA BİBER (*CAPSICUM ANNUUM* L.) TOHUMLARININ *IN VITRO* BÜYÜME VE ÇİMLENMESİ ÜZERİNE DIŞSAL OLARAK UYGULANAN SPERMİN VE PUTRESİNİN ETKİSİ

## *ÖZ*

Bitki büyüme düzenleyicileri tohum çimlenmesinde önemli bir rol oynar. Bu çalışmada tuz stresi altında biberin fide büyümesi (radikula uzunluğu, hipkotil uzunluğu, taze ağırlık, kuru ağırlık) ve tohum çimlenmesi üzerine dışsal poliaminler (spermin ve putresin)'in etkisi araştırılmıştır. Artan tuz konsantrasyonu çimlenme üzerinde önemli bir azalmaya neden olmuştur ($P<0.05$). NaCl hipokotil büyümesini inhibe etmiştir ve bu inhibisyon spermin uygulaması ile engellenmiştir. En iyi çimlenme 2 mM spermin + 200 mM NaCl uygulamasında belirlenmiştir ($P<0.05$). Tek başına Put uygulması çimlenmeyi inhibe etmiştir, fakat hipokotil ve radikula uzunluğu 0.01 mM putresin + 200mM NaCl and 1mM putresin + 50 mM NaCl uygulamaları ile önemli bir şekilde artmıştır ($P<0.05$).

**Anahtar Kelimeler:** Çimlenme, Büyüme, Biber, Putresin, Poliaminler, Spermin

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[1], Ankara University, Faculty of Science, Department of Biology, 06100 Tandoğan, Ankara, TURKEY.
E-posta: ekoc@science.ankara.edu.tr, Tel: +90 (312) 212 67 20/1071/1292
E-posta: ustun@science.ankara.edu.tr

[2], Nigde University, Faculty of Arts and Sciences, Department of Biology, 51240, Nigde, TURKEY.
E-posta: cislek@nigde.edu.tr, Tel: +90 (388) 225 40 57

[3], Ordu University, Faculty of Agriculture, Biometry and Genetics Unit, 52200, Ordu, TURKEY.
E-posta: ykaskoarici@odu.edu.tr

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## 1.INTRODUCTION

Germination is a complex process of plant development, which is set by the balance between levels of plant growth regulators and the temporal and spatial expression of seed-specific gene (Barendse and Peeters, 1995). A part of the embryo, generally the radicle, grows enough to penetrate the surrounding of it and morphologically with the event, germination is completed (Bewley, 1997). Plant growth regulators operate at different levels but play a significant role in germination of seed (Chen and Fankhauser, 2004).

Especially, since the salinisation of agricultural fields occurs due to the irrigation and major important crops are sensitive to salinity of soil, salt stress has been studied comprehensively. Development of plants is limited by enviromental stresses. Among all, salinity is one of the most important environmental stress (Bohnert et al., 1995). Thus, salt stress affects also germination. In addition to this, researches, done up to now, has revealed that some plant growth regulators have a supporting effect on germination of seed.

Polyamines (PAs), which are low molecular weight aliphatic nitrogen compounds, indicate a broad biological activity. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the main PAs, present in higher plants (Kuznetsov et al., 2006; Takahashi and Kakehi, 2010). A broad spectrum of biological activities of PAs are exampled as the regulation of gene expression, signal modulation, cell proliferation, membrane stabilization (Igarashi and Kashiwagi, 2000), cell division and elongation, signalling, root growth, flower and fruit development, plant growth and development to plant adaptation toward abiotic stresses (Silveira et al. 2006; Santa-Catarina et al., 2006; Groppa et al., 2007). As well as the studies that displayed the effect of single PAs alone, other studies combine physiological events in plants with the relationship between two or more PAs (Shoeb et al., 2001). Depending on the type of PAs, its concentration, and the state of the embryo dormancy, exogenous PAs may affect germination of embryos (Farooq et al., 2011).

Processes of plant growth and development are affected by PAs, physiologically (Galston et al., 1997; Applewhite et al., 2000; Tassoni et al., 2000, Kusano et al., 2008). In recent years, involvement of PAs has been concentrated in return for the different environmental stresses (Galston et al., 1997; Bouchereau et al., 1999). Despite the correlation between abiotic stress intensity and PA levels in plants, correlation between them is not completely clear.

The aim of the study was to examine the effects of Spm and Put on seedling growth (radicula elongation, hypocotile elongation, fresh weight, dry weight) and germination of hot pepper seeds.

## 2. MATERIAL and METHODS

In the present study, seeds of KM- Hot pepper (*Capsicum annum* L. ) which were chosen as high quality and had a high germination rate of 100% under optimum conditions were used. The surface of seeds were sterilized with 1 % sodium hypochloride.

Germination experiments were carried out in an incubator at 25º C to prevent germination in continuous dark environment. The seeds, in sufficient amount, were presoaked in 1/2 Hoagland solutions (hereinafter referred to as ‘control’) and in aqueous solutions of NaCl (at three concentrations: 50, 100 and 200mM), Spm (at three concentrations: 0.01, 1 and 2 mM), Put (at three concentrations: 0.01, 1 and 2 mM) or in combinations prepared with NaCl and any two of these regulators, for 24 h at room temparature. For each treatment three specimens, with ten seeds per specimen, were used. Ten seeds from every treatment, were located in 9 cm petri dishes, covered with two sheets of filter paper, and moistened with 20 mL of test solutions. After sowing, petri dishes were put in an incubator for germination in a growth chamber at 25°C for 5 days. The germination percentages of the seeds were examined at $5^{th}$ day. For 10 seeds, the lengths of the radicula and hypocotile in mm, and fresh and dry weights of the seedlings (gr/seedling) were recorded, at the end of experiment ($5^{th}$ day).

## 3. STATISTICAL ANALYSIS

Normalities of distribution of variables were controlled by Kolmogorov-Simirnov Test. Homogeneities of the group variances were controlled by Levene’s Test. One-way ANOVA is the comparison way of the variables, after multiple comparisons between pairs of means were carried out according to the Tukey’s Test. One by one, variables were indicated as mean±standard error of the mean ( $\overline{X} \pm S_{\overline{X}}$ ) and min-max value. ANOVA was implemented by using Minitab 16 software programme and MSTAT package programme was used to compare means, for Tukey's Test. The alpha level was set at %5. At the end of variance analysis; for all properties, concentration differences were significant ($p<0.01$ and $p<0.05$). SNK (Student-Newman-Keuls) test results were displayed as averages by suitable letters within the figures: Difference between averages without common letter was significant ($p<0.05$).

## 4. RESULTS

As anticipated, germination of the seeds was prevented by high salt stress. Our results has revealed that although the difference is not significant, at 50 and 100 mM NaCl treatment, the percentage germination was lower than the control.By comparison with the control, 200 mM NaCl prevented germination and this difference was significant ($p<0.05$) (Fig. 1, 2). In the event of simultaneous application of each PAs and salt stress treatments, it is observed that the percentage of germination increased. At the highest concentration of NaCl (200 mM NaCl), germination of seed was increased by Spm treatment. The best germination was encountered in application of 2 mM Spm + 200 mM NaCl ($p<0.05$). In comparison to control, single applications of Put prevented germination of seed ($p<0.05$) (Fig.1, 2).

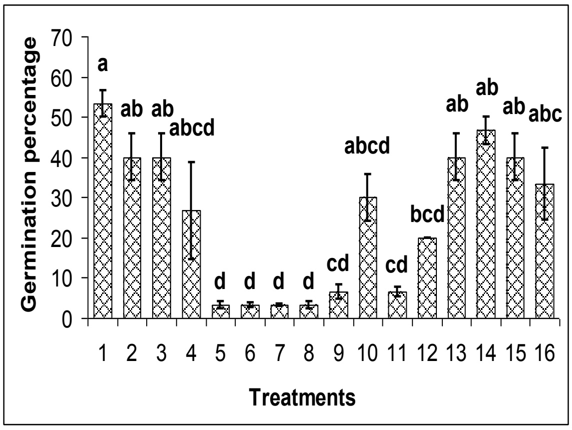


Figure 1. Germination percentage of pepper seeds under salt stress with exogenous added 0.01, 1 and 2 mM of Spm (**1:** Control, **2:** 50 mM NaCl, **3:** 100 mM NaCl, **4:** 200 mM NaCl, **5:** 0,01 mM Spm, **6:** 1 mM Spm, **7:** 2 mM Spm, **8:** 0,01 mM Spm + 50 mM NaCl, **9:** 0,01 mM Spm + 100 mM NaCl, **10:** 0,01 mM Spm + 200 mM NaCl, **11:** 1 mM Spm + 50 mM NaCl, **12:** 1 mM Spm + 100 mM NaCl, **13:** 1 mM Spm + 200 mM NaCl, **14:** 2mM Spm + 50 mM NaCl, **15:** 2 mM Spm + 100 mM NaCl, **16:** 2 mM Spm + 200 mM NaCl ).

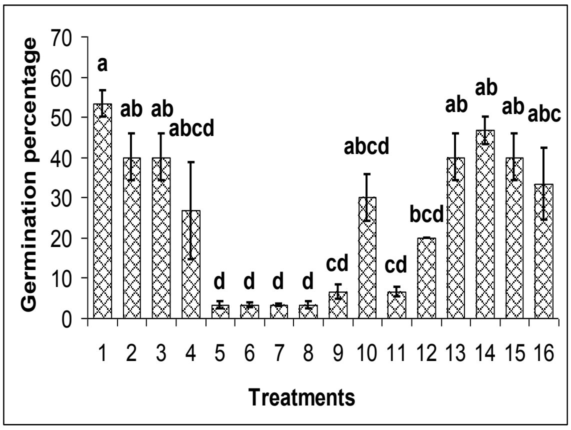


Figure 2. Germination percentage of pepper seeds under salt stress with exogenous added 0.01, 1 and 2 mM of Put (**1:** Control, **2:** 50 mM NaCl, **3:** 100 mM NaCl, **4:** 200 mM NaCl, **5:** 0,01 mM Put, **6:** 1 mM Put, **7:** 2 mM Put, **8:** 0,01 mM Put + 50 mM NaCl, **9:** 0,01 mM Put + 100 mM NaCl, **10:** 0,01 mM Put + 200 mM NaCl, **11:** 1 mM Put + 50 mM NaCl, **12:** 1 mM Put + 100 mM NaCl, **13:** 1 mM Put + 200 mM NaCl, **14:** 2mM Put + 50 mM NaCl, **15:** 2 mM Put + 100 mM NaCl, **16:** 2 mM Put + 200 mM NaCl ).

Fresh and dry weight seedlings were reduced substantially by high salt stress (200mM NaCl), at significant rates ($p<0.05$) (Fig. 3, 4). It is observed that fresh weight increased in low concentration (0.01 m M) of Spm ($p<0.05$) and Put ($p<0.05$) (Fig. 3, 4); in the event of simultaneous application of each PAs and salt stress treatments.

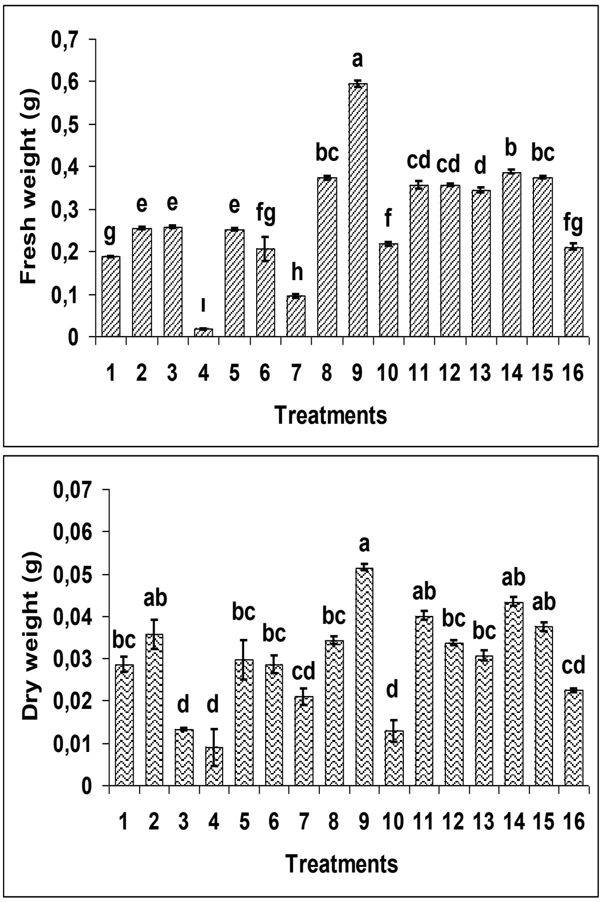


Figure 3. Fresh and dry weight of pepper seeds under salt stress with exogenous added 0.01, 1 and 2 mM of Spm (**1:** Control, **2:** 50 mM NaCl, **3:** 100 mM NaCl, **4:** 200 mM NaCl, **5:** 0,01 mM Spm, **6:** 1 mM Spm, **7:** 2 mM Spm, **8:** 0,01 mM Spm + 50 mM NaCl, **9:** 0,01 mM Spm + 100 mM NaCl, **10:** 0,01 mM Spm + 200 mM NaCl, **11:** 1 mM Spm + 50 mM NaCl, **12:** 1 mM Spm + 100 mM NaCl, **13:** 1 mM Spm + 200 mM NaCl, **14:** 2mM Spm + 50 mM NaCl, **15:** 2 mM Spm + 100 mM NaCl, **16:** 2 mM Spm + 200 mM NaCl.

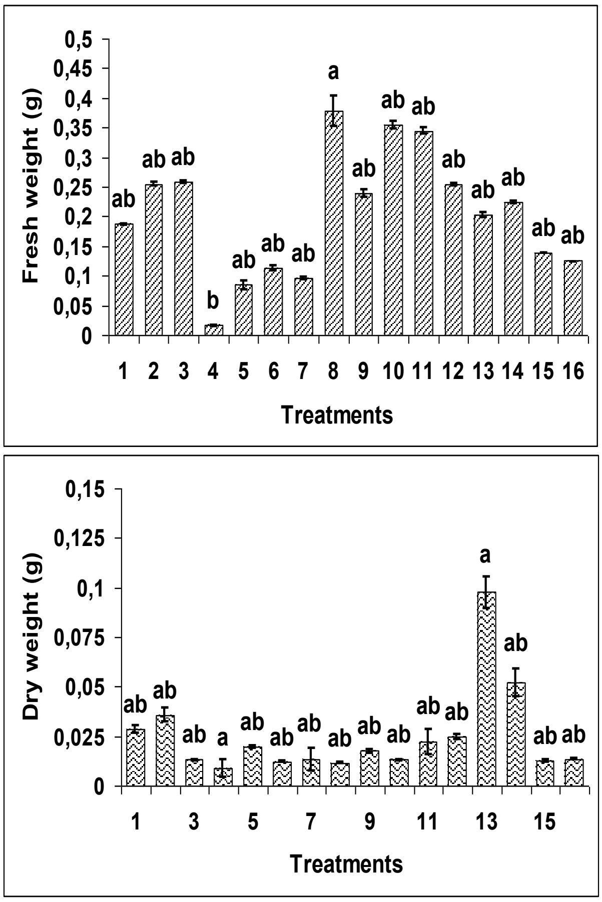


Figure 4. Fresh and dry weight of pepper seeds under salt stress with exogenous added 0.01, 1 and 2 mM of Put (**1:** Control, **2:** 50 mM NaCl, **3:** 100 mM NaCl, **4:** 200 mM NaCl, **5:** 0,01 mM Put, **6:** 1 mM Put, **7:** 2 mM Put, **8:** 0,01 mM Put + 50 mM NaCl, **9:** 0,01 mM Put + 100 mM NaCl, **10:** 0,01 mM Put + 200 mM NaCl, **11:** 1 mM Put + 50 mM NaCl, **12:** 1 mM Put + 100 mM NaCl, **13:** 1 mM Put + 200 mM NaCl, **14:** 2mM Put + 50 mM NaCl, **15:** 2 mM Put + 100 mM NaCl, **16:** 2 mM Put + 200 mM NaCl).

Roots elongated to 7.49 mm under control condition, while under high salt stress condition (200 mM NaCl), root growth was averaged to 12.908 mm, meaning a 72.33% increase in root growth ($p<0.05$) (Fig. 5, 6). Increasing salinity significantly decreased hypocotile growth. Under high salt stress, hypocotile growth was reduced by 85% compared to controlled condition ($p<0.05$). Under 0.01 mM Spm +100 Mm NaCl and 1mM Put + 50 mM NaCl combinations ($p<0.05$) (Fig. 5, 6); hypocotile and root growth of seedling significantly increased. The lowest value of root and hypocotile growth was in 2mM Spm treatment (Fig. 5).

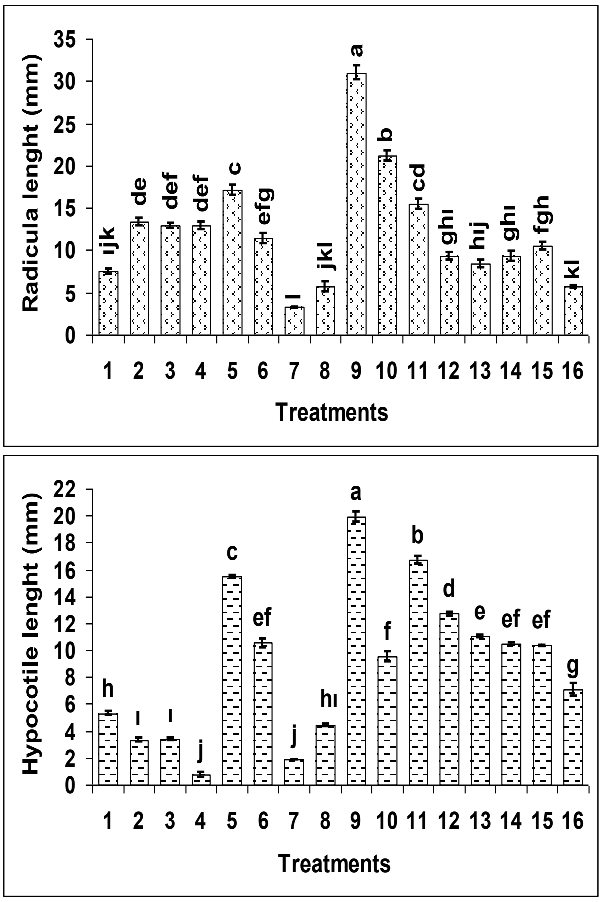


Figure 5. Radicula and hypocotile lenght of pepper seeds under salt stress with exogenous added 0.01, 1 and 2 mM of Spm (**1:** Control, **2:** 50 mM NaCl, **3:** 100 mM NaCl, **4:** 200 mM NaCl, **5:** 0,01 mM Spm, **6:** 1 mM Spm, **7:** 2 mM Spm, **8:** 0,01 mM Spm + 50 mM NaCl, **9:** 0,01 mM Spm + 100 mM NaCl, **10:** 0,01 mM Spm + 200 mM NaCl, **11:** 1 mM Spm + 50 mM NaCl, **12:** 1 mM Spm + 100 mM NaCl, **13:** 1 mM Spm + 200 mM NaCl, **14:** 2mM Spm + 50 mM NaCl, **15:** 2 mM Spm + 100 mM NaCl, **16:** 2 mM Spm + 200 mM NaCl.

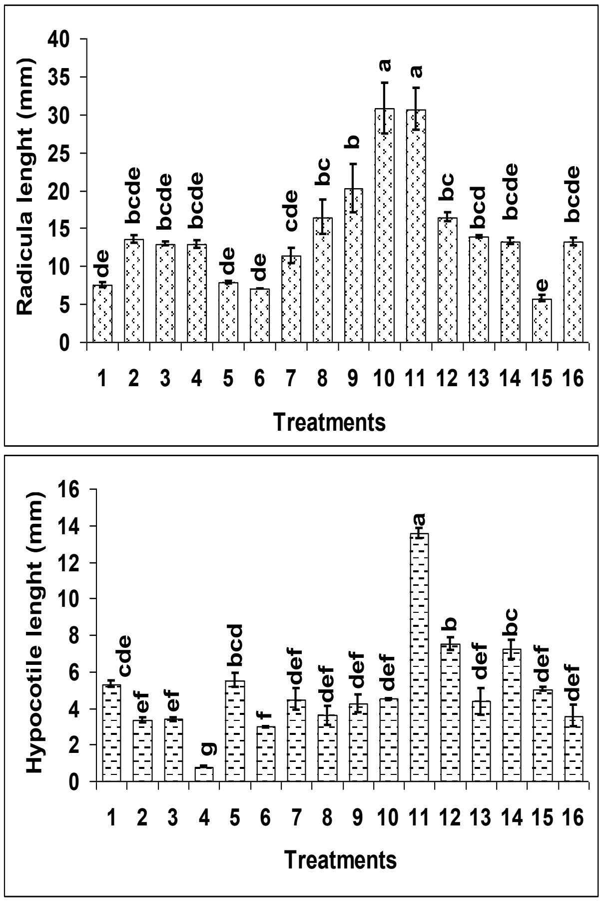


Figure 6. Radicula and hypocotile lenght of pepper seeds under salt stress with exogenous added 0.01, 1 and 2 mM of Put **(1:** Control, **2:** 50 mM NaCl, **3:** 100 mM NaCl, **4:** 200 mM NaCl, **5:** 0,01 mM Put, **6:** 1 mM Put, **7:** 2 mM Put, **8:** 0,01 mM Put + 50 mM NaCl, **9:** 0,01 mM Put + 100 mM NaCl, **10:** 0,01 mM Put + 200 mM NaCl, **11:** 1 mM Put + 50 mM NaCl, **12:** 1 mM Put + 100 mM NaCl, **13:** 1 mM Put + 200 mM NaCl, **14:** 2mM Put + 50 mM NaCl, **15:** 2 mM Put + 100 mM NaCl, **16:** 2 mM Put + 200 mM NaCl).

## 5. DISCUSSION

Many physiological activity of plant growth is affected by salt stress. Because of the inhibitory effect of salt on cell division and enlargement in growing point, shoot growth was reduced by salinity. Early flowering reduced dry matter, increased root: shoot ratio. And under salt stress condition, leaf size, caused by salinity, may be considered as possible ways of decreasing yield in wheat seedlings (Mass and Poss, 1989). Toxic influences of salt may affect hormonal balance of plants.

In order to increase stress tolerance under stress conditions, PAs are used in plants, successfully. In recent years, molecular, biochemical and physiological effects of large PA molecules have been examined. Implementation of PA inhibits growing and cell division inhibition created by stress, externally. This indicates the importance of PAs in plant development. Therefore, alternative approaches including PA implementations, may increase the tolerance to the various stresses. Besides; data, obtained, may give way to implemantation to different species.

Germination of seed is prevented by salinity via osmotic stress (Abdoli et al. 2013). About delay and decrease of germination in various plants under salt stress, there are many researches. α-amylase is the most important enzyme in seed germination inductance that its action is affected by salt stress. Na bonds, in order to active site of this enzyme, result in decrease in activity and destroy in the structure of this enzyme (Saboury and Karbassi, 2000). According to studies salt stress prevents germination of seeds. Spm induced the seed germination but Put was not found effective.

Khan et al. (2012) have discovered that 25, 50, 75 and 100Mm Spm and Spd stimulate germination pepper which is not affected by any stress. On the other hand, they found Put efficient in low concentrations. In their study, they mentioned that Put is more efficient than Spm, Spd in germination whereas in our study, Spm has given more positive results in germination.

As a result of the studies, salt stress prevents hypocotile growth more than root growth (Campestre et al. 2011). Similar results were found by Huang and Reddman (1995), Foolad (1996), Keiffer and Ungar (1997) and Jeannette et al. (2002). Inhibitory effects of salt stress on root and shoot growth increased root therefore shoot ratio. In that case, this may have the advantage of increase in ratio of water absorption to transpiration area which is useful for dry land condition (Moudi and Maghsoui, 2008).

Pretreatments of seeds are chemical and physical mediums to secure rapid and uniform germination of seed. Our study introduced that in low concentrations of PAs, pre-treated seeds of pepper can increase hypocotyls-radicle length. Spm + NaCl combinations and Put + NaCl combinations at low concentration increased hypocotyls- radicle length.

According to the study, application of Spm increased hypocotyle length in pepper plant. The reason of rapid germination of pepper seed may be the increase of endogenous GA and IAA and the decrease of ABA content. Pierruzzi et al. (2011) explained that Spm and Put ratios may be used as markers for germination completion. And before the germination, increase in IAA levels may be related with variations in PAs content. Bagni (1970) mentioned that Spm and Spd are synthesized and translocated during germination of bean (*Phaseolus vulgaris*). The greater part of the PAs was transferred from cotyledons into the different part of the growing seedlings. Our study showed that at different concentrations, Spm substantially implemented differential influence on fresh and dry weight of seedlings. Toxic effects of salts may be reduced by lower concentration of Spm and Put (specially 0.01mM) in PAs-NaCl combination. This may lead to an increase in fresh and dry weight of the seedlings. This effect of Put on fresh and dry weight of seedlings are similar to the results obtained by Wahed (2006) in cotton plant and Nassar et al. (2003) in *Phaseolus vulgaris* L.

Indirect results are obtained from some researches. It is well accepted that uptake of nutrients and water by fine roots is efficient (Strand et al., 2008). In this research, uptake of nutrients and water by the root may be the reason of the increased hypocotyls and radicle length by pre-soaking seeds in PAs. Also, polyamines could act as source of nitrogen, which supports growth (Smith, 1982).

Based on our studies, on both fresh and dry weights, high concentration levels of NaCl (200mM) in the growth medium resulted in a markable reduction in growth. In many plants, such an unfavorable effect of salt stress on growth has been observed. This reduction in growth is suggested to be related to the restraint of the synthesis of growth regulators such as cytokinine and the increase in the production of the inhibitors such as ABA (Khan et al., 1976; Ungar, 1991) .

## 6. CONCLUSION

Our results have revealed that specifically 0.01mM Spm and 0.01mM Put and 1mM Put activated the seedling growth via PAs, involving in protection from salt stress. And pre-treatment is a useful method to increase growth in pepper seeds, but Put wasn't effective on germination, literally. All the concentrations of Spm were found better as compared to Put. For normal growth and germination of pepper seeds, the results indicated that Spm is required.

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